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Relocation sensors to quantify signaling dynamics in live single cells

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All cells are different. Even isogenic cells can possess diverse shapes, reside in different cell-cycle stages or express various sets of proteins. These variations can modulate the cell response to environmental stimuli and thereby provide key insights into the regulation of signal transduction cascades. Fluorescence microscopy allows to visualize these differences and monitor in real-time the responses of live single cells. In order to observe key cellular events, fluorescent biosensors have been developed. Among many assays, relocation reporters play an important role since they enable the quantification of the signal transduction dynamics. Fluorescently tagged endogenous proteins, as well as synthetic constructs, have allowed the measurement of kinase activity, transcription factor activation, transcription and protein expression in live single cells.

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Introduction

Cells are the fundamental units of every biological organism. There is on the order of thirty trillion cells in the human body that are all descendants of a single fertilized egg. Through series of divisions and differentiation events, each one of these cells has diverged from this founding cell and is now different from its neighbors. Because of different environment, cell size, shape and protein content, it is thus highly unlikely to find two identical cells in a human body. Even in an isogenic population of unicellular organisms such as *Escherichia coli* or *Saccharomyces cerevisiae*, these inherent physiological variations will render each cell unique.

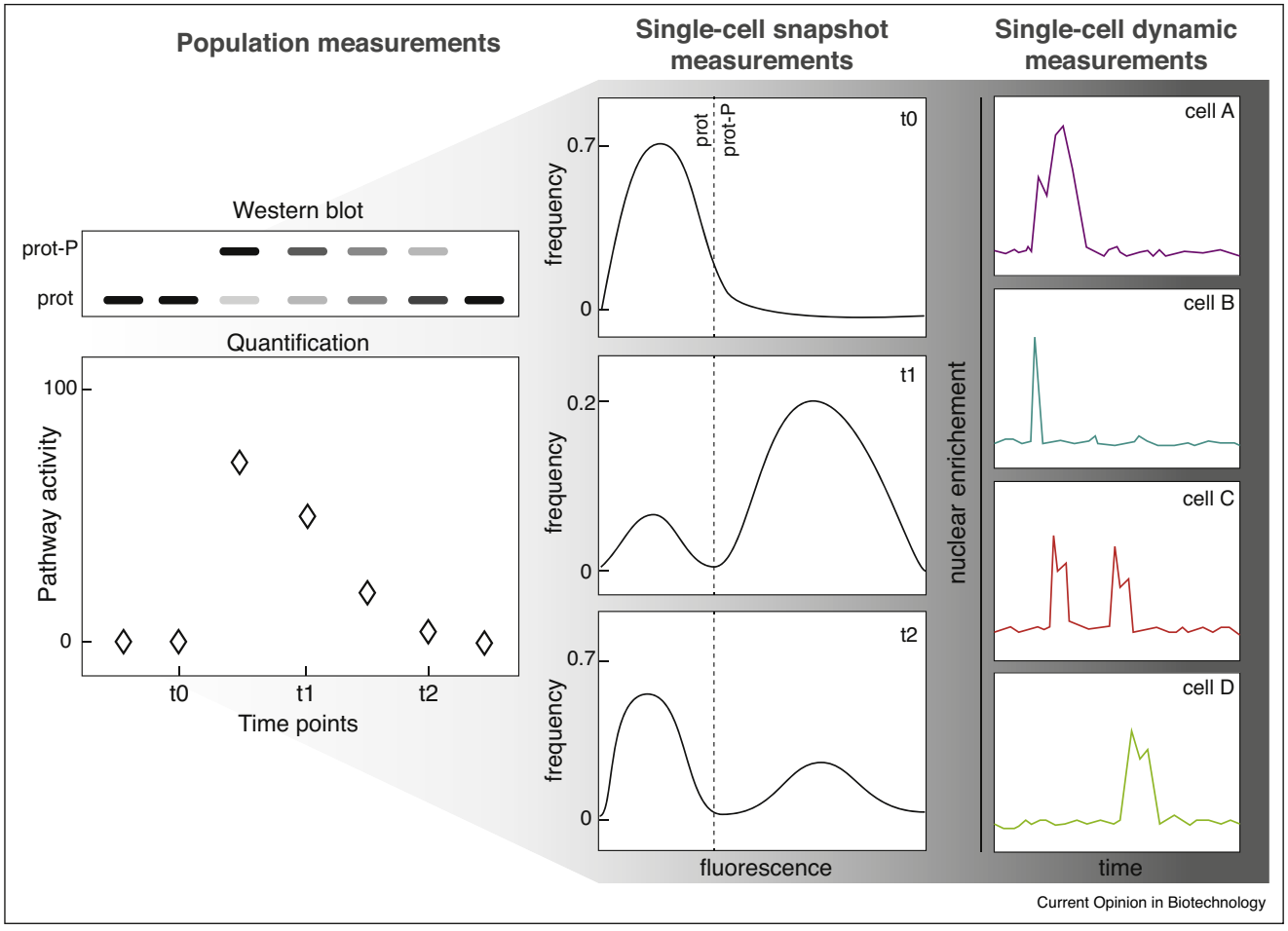
It is hence crucial to understand how this variability can influence the fate of a cell. How do cellular differences lead to a robust response? How diverse can this response be, to allow the right amount of plasticity favoring evolution of the system while enabling robust cellular adaptation?

In the last decades, the notions of biological noise and cellular variability have gained in importance, with seminal papers from Ferrell and Machleder [1] or Elowitz *et al.* [2]. In the signal transduction field, these concepts have deep implications: understanding why and how a population of cells can respond in a uniform fashion to a stimulus, while these same cells can display a bimodal behavior in other situations, can provide valuable insights into the regulation of the investigated pathways. Indeed, the single-cell behavior bears the underlying signature of the molecular mechanisms that compose the signal transduction cascade. Single-cell measurements can thus yield crucial information on the rich interconnections of signaling pathways that are made of a complex network of positive and negative feedback or feedforward loops.

In order to monitor the diversity in the cellular responses to a stimulus, flow cytometry [3] and microscopy [4] measurements have become more and more prevalent. Additionally, novel techniques such as single-cell transcriptomics [5] and mass cytometry [6] have been developed. All these tools have provided key insights into the characterization of the signaling response of individual cells.

Although microscopy is by far the oldest single-cell technique [7], it continues to play a major role in deciphering the response of individual cells. Indeed, the development of fluorescent proteins [8,9•] has provided new means to directly probe the abundance and location of proteins in live single cells under various conditions. This breakthrough promoted the development of live-cell imaging, allowing the measurement of living specimens spanning a few minutes to multiple days [10,11•]. Whereas, traditional biochemical assays only provide a snapshot of the mean response of a population of cells at a given time point, single-cell measurements have gone beyond this average by revealing the diversity among cells of the same population (Figure 1). In addition, microscopy possesses the unique ability to observe the same cell over time allowing to monitor the dynamic changes occurring within a given cell. Thus, following an individual cell across time enables the correlation of its initial state (cell-cycle stage,

Figure 1



Dynamic single-cell measurements provide essential insights into the regulation of a signaling cascade. Population level measurements, such as western blots quantifying the phosphorylation level of a protein prot-P vs prot, report on the activity of a cellular component at various time-points after a stimulus. The outcomes are thus discrete measurement of a large number of cells (diamonds plot). Snapshot single-cell measurements, for instance from flow cytometry experiments, provide insights into how this activity is distributed between different cells in the population. In this example, after stimulus, only a fraction of the population is found in the active state while the other part remains inactive (histogram plots). These measurements indicate how these two states are populated at different times. Only dynamic single-cell measurements allow to follow in real-time the response of the cell and monitor all its transitions between the active and inactive states, revealing the true dynamic of the signaling cascade activation (time-course traces).

expression capacity, age, etc.) with its signaling response and the long-term biological outcome induced by the signaling cascade.

Transcriptional changes or cell-cycle progression can require multiple hours to take place. However, signal transduction dynamics can happen within tens of seconds to a few minutes. Monitoring these fast cellular changes requires biosensors that can act at these time scales. Researchers in the signaling field have often relied on protein relocation to follow the dynamic activation of signal transduction cascades.

Relocation sensors

In this review, we will distinguish three different classes of protein relocation-based assays. First, we will introduce endogenous relocation reporters, which consist in endogenous proteins tagged with a fluorescent protein. Then, two kinds of synthetic relocation reporters will be described: passive and active biosensors. Passive biosensors bind to the active form of a protein of interest and thus allow to monitor its localization and/or its level. Active relocation sensors are direct substrates of the investigated enzyme and the subcellular location of the sensor is controlled by the activity of this protein.

Endogenous relocation reporters

Endogenous relocation reporters are the most prevalent type of biosensors present in the literature. With the emergence of protein tagging by fluorescent proteins, researchers quickly noticed that some of them had distinct subcellular localizations depending on their activity